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Effect of ethanol on structural transitions of DNA and polyphosphates under Ca^{2+} ions action in mixed solutions★

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In the present work using the IR spectroscopy method the effect of ethanol on structural transitions of DNA and polyphosphates under the action of Ca^{2+} ions in mixed solutions containing ethanol (0–25 vol.%) was studied.

It was shown that, on its interaction with Ca^{2+} ions, in aqueous and mixed solutions DNA becomes transformed into compact form. With the increase of concentration of ethanol the degree of Ca^{2+}-induced DNA compactisation rises. It was found that, in mixed solutions containing ethanol, Ca^{2+}-induced DNA compactisation depends not only on the solution's dielectric permeability but also on the solution structure. On stabilisation of the water structure in the presence of low ethanol concentrations a stabilisation of the DNA macromolecule occurs that leads to the increase of the Ca^{2+} ion concentration necessary for DNA compactisation.

Comparison of the effects of ethanol on Ca^{2+}-induced structural transitions in DNA and polyphosphates in mixed solvents permits to suppose that at alcohol concentrations in solution resulting in disruption of the water spatial structure, some peculiarities are observed in the behavior of those molecules whose hydrophobic interactions are essential.

Studies on DNA interaction with metal ions are of great interest because of the main role metal ions play in functioning of the genetic apparatus *in vivo*. Along with water, metal ions stabilize the structure of nucleic acids, control the equilibrium between different forms of secondary and tertiary structures, take part in processes of DNA transcription and replication.

Moreover, recently many medicines (first of all antibacterial and antiviral ones) have been developed that use nucleic acids – DNA and RNA – as their targets [1, 2]. Most of these antibiotics are in ionic form and bind to DNA by

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Abbreviation: pP, polyphosphate.

the cationic mechanism. Therefore, DNA complexes with metal cations can be applied as model systems in studies on the action of such substances on the DNA structure.

DNA interaction with Ca^{2+} ions in solution and in films have been much investigated in literature [3–16]. Binding sites of Ca^{2+} ions on DNA and binding constants have been determined [3–10, 15, 16]. It was shown that over a wide concentration range Ca^{2+} ions bound preferentially to oxygen atoms of DNA phosphate groups in the ratio of one ion per two phosphate groups. The effect of Ca^{2+} ions, together with that of other divalent metal ions, on DNA conformational transitions, first of all on B-A and helix-coil transitions, were also extensively studied [9, 11–14]. In particular, it has been shown that for DNA complexes with Ca^{2+} ions in films the transition from the disordered to B-form occurs without passing through the A-form (except the complexes with $[Ca^{2+}]/[P] = 20$), and the number of water molecules per nucleotide is higher; this means that Ca^{2+} ions delay DNA transition into the B-conformation [9, 14]. Ca^{2+} ions increase as well the DNA melting temperature [13].

So far, little experimental evidence is available for structural transitions in the DNA macromolecule induced by divalent metal ions, including Ca^{2+}. In our previous works we have shown that the Ca^{2+} ion interaction with DNA in aqueous solution of high DNA concentration is able to induce DNA structural transition [15, 16]. When comparing DNA complexes with Cu^{2+}, Ca^{2+} and Tb^{3+} ions, we have shown that this structural transition of DNA is similar to the DNA transition into the compact state [17].

In the present work we study the effect of low (up to 25 vol.%) ethanol concentrations on Ca^{2+}-induced DNA structural transitions. It is known that in mixed solutions containing alcohols DNA compactisation can be induced by divalent metal ions [18, 19]. However, in the works studying such DNA compactisation, higher alcohol concentrations were used ([18, 19] and references therein [20–22]). Votavova *et al.* [22] showed that, up to 40 vol.% of methanol, the addition of divalent ions led to DNA stabilization (i.e. to increase of DNA melting temperature); only at methanol concentration higher than 50 vol.% the presence of divalent cations caused DNA condensation and denaturation.

MATERIALS AND METHOD

Native calf thymus DNA of molecular mass 1.9×10^7 Da, protein content lower than 0.1%, RNA content lower than 0.2%, hypochromic effect of 36% and turbidity (D_{320} for DNA concentration of 1 mg/ml) lower than 0.025 o.u., was used. The amount of Na^+ and K^+ ions in relation to DNA dry mass, determined with an FPL-1 flame photometer, was 7.0 ± 0.2% and 0.6 ± 0.2%, respectively. DNA was extracted in Prof. D. Lando's laboratory (Institute of Bioorganic Chemistry, Academy of Sciences of Belarus Republic). Methods of isolation and purification of DNA samples have been described in detail by Lando *et al.* [23].

The polyphosphates used $Na_{n+2}P_nO_{3n+1}$ (type 65) with average chain length of 65 ± 5 P were from Sigma.

DNA and polyphosphate samples were dissolved in cacodylate buffer, Na^+ concentration being 5×10^{-3} M, pH 7 ± 0.1. The biopolymer concentration in solution determined by UV spectroscopy was in the range of $(3.9–5) \times 10^{-2}$ M P.

Double-rectified ethanol was used in the work. The purification and percentage content of ethanol were controlled spectrophotometrically and by the index of dielectric permeability. To characterise quantitatively the nonelectrolyte content, the ethanol volume concentration (vol.%) minus the nonelectrolyte volume in 100 volumes of solution, was used.

Infrared spectra of DNA complexes with Ca^{2+} ions in solution were recorded by the in-

frared spectrophotometer UR-20 (Karl Zeiss, Jena, Germany). The spectral split width was 6 cm^{-1} (at 1400 cm^{-1}), the rate of the spectrum registration being 10 cm^{-1}/min. The absolute error of wave number was ± 1.5%. To take spectra, special CaF_2 cuvettes with path length of 62 μm were used. The cuvettes were thermostated at 29°C, the temperature being controlled by the thermocouple with an accuracy of ± 0.1°C.

The optical density D (with an accuracy of 2%) was determined by the base line method, the value of D was set as the base line on the building of the absorption spectra at the frequency ν = 1450 cm^{-1} at which absorption bands of DNA were absent.

RESULTS AND DISCUSSION

IR spectra of DNA and DNA complexes with Ca^{2+} ions in aqueous and mixed solutions containing ethanol (0–25 vol.%) were recorded in the absorption region of DNA phosphate groups (1000–1400 cm^{-1}).

IR spectra of DNA in aqueous and aqueous-ethanol solutions (Fig. 1) have 3 main absorption bands at 1053 cm^{-1} (vibrations of the C–O–P sugar–phosphate backbone [24]), 1090 cm^{-1} (ν_S – symmetrical vibrations of phosphate groups [24]) and 1223 cm^{-1} (ν_{aS} – asymmetrical vibrations of phosphate groups [24]). The positions of these bands and the ratio of their intensities correspond to the IR spectrum of the native DNA in B-form in solution [15, 16, 24]. It should be noted that for DNA in 25% ethanol solution without Ca^{2+} ions, band shifts were observed: 1053 → 1056, 1223 → 1225 cm^{-1}. Thus, a partial destabilisation of the DNA structure took place in this solution.

In IR spectra of DNA complexes with Ca^{2+} ions in aqueous-ethanol solutions absorption bands shifted to higher frequencies: 1090 → 1094–1096, 1053 → 1057–1058, 1223 → 1226–1227 cm^{-1}, like those of DNA complexes with Ca^{2+} ions in aqueous solution [15, 16]. The general character of changes in spectra on the formation of DNA metal complexes in mixed solutions with different ethanol content was similar. With the increase of the ethanol content in the mixed solution the Ca^{2+} ion concentration that induces changes in IR spectra of DNA–Ca^{2+} complexes became lower. The absorption band shifts observed evidence the Ca^{2+} ions binding to DNA phosphate groups in aqueous-ethanol solution. The marker band of the DNA B-form, present in spectra of DNA complexes with Ca^{2+} ions (excluding complexes found at high concentration of calcium ions) at 1223 cm^{-1} [24] as well as the band at 1053 cm^{-1} indicating the double helical state of DNA, together with the absence of significant shifts of these bands, evidence that DNA complexed with Ca^{2+} ions in aqueous-ethanol solutions remains in the range of B-conformation. The exceptions are

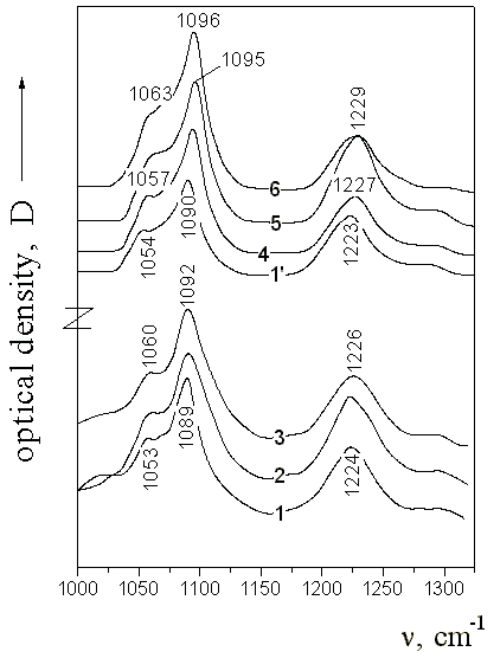

Figure 1. The IR-spectra of DNA (1, 1') and DNA complexes with Ca^{2+} ions in aqueous (1–3, [16]) and mixed solution containing 15 vol. % of ethanol (1', 4–6).

Ca^{2+} ion concentrations, M: 4.32 × 10^{-1} (2), 8.68 × 10^{-1} (3), 6 × 10^{-2} (4), 10^{-1} (5); 1.3 × 10^{-1} (6). Concentration of Na^+ ions – 5×10^{-3} M, DNA concentration (4.2–4.8) × 10^{-2} M P, temperature 29°C, pH 7.

DNA complexes with high concentrations of Ca^{2+} ions. In this case strong shifts of absorption bands (1090 → 1100, 1053 → 1060–1065, 1223 → 1228–1230 cm^{-1}), followed by the band broadening and by an increase of the background scattering, support significant disordering and aggregation/precipitation of the DNA complex with high concentrations of Ca^{2+} ions. To study such complexes was beyond the scope of the present work.

Besides, as Fig. 1 shows, due to the DNA interaction with Ca^{2+} ions in mixed solutions, as in the case of DNA–Ca^{2+} ion complexes in aqueous solution, the intensities of absorption bands of symmetrical and asymmetrical vibrations of DNA phosphate groups increased sharply. It should be noted that the increase of the peak intensity of IR absorption bands was followed by a proportional rise of the integral intensity of these bands.

Changes IR spectra of DNA on its interaction with Ca^{2+} ions in aqueous-ethanol solutions are similar to changes observed by us in IR spectra on formation of DNA–Ca^{2+} complexes in aqueous solution [15, 16]. In our previous works [15–17] we attributed these changes in IR spectra and, first of all, the observed sharp increase of absorption band intensities, to the DNA transition into the compact state under the action of divalent metal ions in aqueous solution. Comparison of results of the above work and of the present one permits to conclude that, on Ca^{2+} ions binding to DNA in mixed solutions containing ethanol, DNA undergoes a transition into compact state too, remaining in B-form.

Figure 2 shows the dependence of the relative change in intensity (R) of absorption bands of symmetrical and asymmetrical vibrations of DNA phosphate groups on the total concentration of Ca^{2+} ions in solution ([Ca^{2+}]) for DNA complexes with Ca^{2+} ions in aqueous and mixed solutions containing ethanol. The dependence R([Ca^{2+}]) for DNA complexes with Ca^{2+} ions in mixed solutions containing ethanol is close to R([Ca^{2+}]) for complexes in the aqueous solution: with the increase of the Ca^{2+} ions concentration the intensity of absorption bands rises. At a definite concentration of Ca^{2+} ions the dependence R([Ca^{2+}]) tends to a plateau. In solution of high ethanol concentrations (15–25 vol.%), on further increase of the Ca^{2+} ion concentration, a decrease of R is observed, corresponding to aggregation and partial precipitation of DNA in the complex with high concentrations of Ca^{2+} ions. We have shown previously [15–17] that the increase of absorption band intensities observed (and, as a result, a rise of value R) is due to the DNA transition into compact state under the action of divalent metal ions. As for DNA–Ca^{2+} complexes in aqueous solution, in aqueous-ethanol solution the value of R increases over a rather narrow interval of Ca^{2+} ion concentrations (Fig. 2) that evidences the positive cooperativity of the DNA compactisation process.

Comparison of results of the present work with the data on DNA compactisation under the action of Cu^{2+} ions both in aqueous and in aqueous-ethanol solutions presented in our

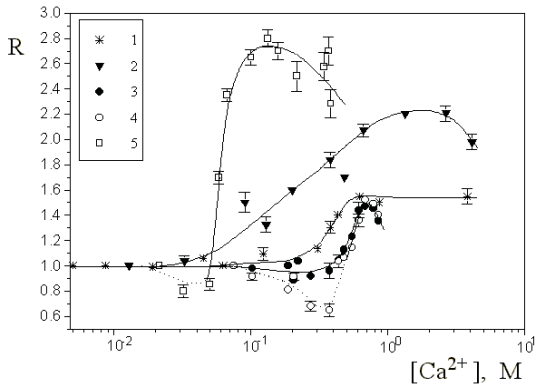

Figure 2. Dependence of relative change in intensity (R) of absorption bands ν_S (1, 2, 3, 5) and ν_{aS} (4) on total Ca^{2+} ions concentration in solution, for DNA complexes with Ca^{2+} ions in aqueous (1) and in mixed solutions containing ethanol at: 4 (2), 9 (3, 4), or 15 (5) vol.%.

$R=D_i/D_0$, where D_0 – the optical density at the maximum of absorption band at given frequency for DNA without divalent metal ions, D_i – the same value for DNA complex with divalent metal cation. Concentration of Na^+ ions – 5×10^{-3} M, DNA concentration $(4.2–4.8) \times 10^{-2}$ M P, temperature 29°C, pH 7.

previous works [15, 16], permits to conclude that Cu^{2+}-induced DNA compactisation is of rather more pronounced character and of higher positive cooperativity and occurs at a rather lower concentration of divalent metal ions than Ca^{2+}-induced DNA compactisation. These differences can be explained by differences in binding constants of Cu^{2+} and Ca^{2+} ions interacting with DNA [10, 12, 13].

With the increase of the ethanol concentration in the mixed solution the R_{max} value (i.e. the amplitude of the rise of the R value) increases (Fig. 2). As we attribute the increase of the intensity of absorption bands in our experiments to DNA compactisation on its interaction with Ca^{2+} ions, the rise of the value R_{max} may be due to the increase of the DNA compactisation degree and/or the density of the DNA packing in compact particles, or due to the change in the form of DNA compact particles formed in solutions with different ethanol content (it was shown [20] that in mixed solutions containing single-atom alcohols the form of DNA compact particles changed from toroids to rods with the decrease of the solution dielectric permeability).

As may be seen from Fig. 2, the Ca^{2+} ion concentration necessary for DNA compactisation depends on the ethanol content in the mixed solution. To characterize it quantitatively, we use in the present work two values: C_{init}, the initial (threshold) value of the Ca^{2+} concentration at which the value R begins to increase, and $C_{1/2}$, the Ca^{2+} ion concentration at which the value R is equal to the half R maximum for the dependence R([Ca^{2+}]): $R(C_{1/2}) = R_{max}/2$. Dependence of values C_{init} and $C_{1/2}$ on the ethanol concentration in the mixed solution is of non-monotonous character (Fig. 3): low ethanol concentrations (up to 5–10 vol.%) induce the increase of C_{init} and $C_{1/2}$; with the rise of ethanol concentrations up to 15–25 vol.%, C_{init} and $C_{1/2}$ decrease. Thus, in mixed solutions containing 5–10 vol.% ethanol a higher Ca^{2+} ion concentration is required for DNA transition into the compact state than is required in the aqueous solution. With the further increase of the ethanol content in the mixed solution, the Ca^{2+} ion concentration required for DNA compactisation decreases.

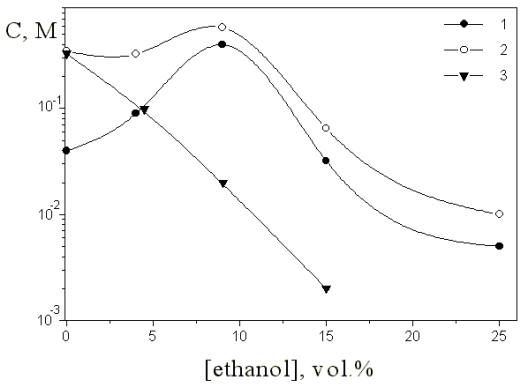

Figure 3. Dependence of C_{init} (1, 3) and $C_{1/2}$ (2) on volume ethanol concentration in mixed solution for the DNA (1, 2) and polyphosphates (3) complexes with Ca^{2+} ions in mixed solutions containing ethanol.

C_{init} – the initial (threshold) value of the Ca^{2+} concentration at which the DNA and polyphosphates structural transitions begin, $C_{1/2}$ – the Ca^{2+} ions concentration at which the R value is equal to the half R maximum for the present dependence R([Ca^{2+}]): $R(C_{1/2}) = R_{max}/2$. Experimental conditions are the same as in Figs. 1 and 2.

It is known that electrostatic repulsion of negatively charged phosphate groups is one of the main barriers preventing DNA compactisation. Therefore, for DNA transition into compact state, it is necessary to neutralize a part of charges on phosphates at the expence of counterions binding to DNA [18, 19]. According to Wilson & Bloomfield [21] and Manning [25], the fraction of the charge neutralized on the polyelectrolyte binding to counterions of Z valence is equal to $\theta = 1 - \frac{1}{Z\xi}$, where $\xi = q^2/\varepsilon T k_B b$, ε is the dielectric constant of the solvent, T is the Kelvin temperature, k_B is the Boltzman constant, b is the average charge density along the DNA backbone before condensation. Thus

$$\theta = 1 - \frac{1}{Z\xi} = 1 - \frac{\varepsilon \times T \times k_B \times b}{Z \times q^2} \approx 1 - A \times \varepsilon,$$

where A is a numerical coefficient (A > 0). Wilson & Bloomfield [21] have shown that for DNA transition DNA into compact state, 89–90% of the total charge on phosphates must be neutralized. This value is the same for aqueous and aqueous-ethanol solutions [20], as well as for phage DNA having the charge density different from that of calf thymus DNA ([18] and references therein). With the decrease of the solvent dielectric permeability ε, due to addition of less polar ethanol, the value θ will increase. In this case some additional condensation of counterions on DNA will occur lowering the surface charge density. The last fact means that, with the decrease of the solution value, the degree of the charge neutralization on phosphates necessary for DNA compactisation will be reached at a smaller counterion concentration. Thus, in accordance with the earlier cited reports [18, 21], the Ca^{2+} ion concentration required for DNA compactisation in aqueous-ethanol solutions must decrease with the increase of the volume ethanol content in solution. As the solution ε value decreases monotonously with the increase of the ethanol content in the mixed solution in the ethanol concentration range studied, it should be expected that the Ca^{2+} ion concentration required for DNA compactisation (dependent on the value θ) will increase monotonously too. The observed non-monotone character of the C_{init} and $C_{1/2}$ dependence on the ethanol concentration in the mixed solution (Fig. 3) permits to suppose that Ca^{2+}-induced DNA compactisation studied in the present work depends not only on the solution dielectric permeability.

In addition to the dependence on the solution dielectric permeability, Ca^{2+}-induced DNA compactisation has to depend on the solution structure because, in the ethanol concentration range studied in the present work, the physical properties of aqueous-alcohol solvents depend in a complex way on the alcohol concentration [26, 27]. It is known that, in the region of low alcohol concentrations, the tetrahedral structure of pure water is retained and alcohol molecules become incorporated into cavities in the water structure and induce either stabilisation of local formations or their rebuilding in order to strengthen the water structure. This is followed by a decrease of the self-diffusion coefficient, dielectric relaxation of water molecules, with extreme apparent in "content-property" diagrams. The stabilising effect of alcohol reaches its maximum value at a definite alcohol concentration. The subsequent increase of the alcohol concentration leads to the disruption of the ordered spatial structure of water and, as a result, to the formation of non-water component associates maintained by hydrophobic interactions.

In the mixed solutions containing additions of different single-atom alcohols at the definite alcohol concentrations (approximate conc.: methanol, 12 mol.%; ethanol, 7 mol.%; n-propanol, 5 mol.%; n-butanol, 1.7 mol.%) the intrinsic viscosity ([η]) of the DNA solution decreases and the more [η] decreases the lower is the solution ionic strength [28, 29]. With the rise of the number of carbon atoms in the alcohol molecule, the alcohol concentration resulting in the drop of [η] becomes low. The work of Veselkov & Frisman [29] shows that the decrease of the DNA [η] and the beginning of the structural transition in the solvent occur at the same alcohol concentration. Frisman and coworkers [28, 29] concluded that the observed dependence of the DNA [η] on the alcohol concentration in the solvent resulted from conformational transitions in the tertiary structure of the macromolecule due to changes in strengths of long-range interaction in the DNA molecule. Those changes occur due to structural rebuilding of DNA in the mixed solvent [28, 29].

Thus, a small volume of ethanol added to the DNA solution, resulting in stabilisation of the water structure, induces conformational transitions in the DNA tertiary structure. In this case, the DNA intrinsic viscosity and, as a result, mean-square dimensions of the DNA coil decrease. As the present work shows, in the range of ethanol concentrations[1] studied the

Ca^{2+} ion concentration required for DNA compactisation increases. Thus, a conclusion may be derived that stabilisation of the DNA macromolecule in the region of low ethanol concentrations in the mixed solution results in the increase of the Ca^{2+} ion concentration required to induce DNA compactisation. So, DNA compactisation depends not only on the dielectric permeability but on the solution structure as well. On stabilisation of the water structure in the presence of low ethanol concentration the Ca^{2+} ion concentration necessary for DNA compactisation increases.

Stabilisation of the DNA double helix in the solution with the about 10 vol.% ethanol concentration can be evidenced by the decrease of intensities of absorption bands of phosphate groups of DNA complexed with Ca^{2+} ions (that is by the decrease of the value R, preceding the rise of this value with the increase of the calcium ion concentration, Fig. 2). The similar decrease of the intensity of absorption bands in FTIR-spectra of DNA metal complexes was demonstrated by Tajmir-Riahi *et al.* [30] who attributed this decrease of the intensity simply to stabilisation of the double helix on the metal (Cu^{2+}) binding to DNA phosphate groups.

It has been shown in a number of works that relatively small additions of single-atom alcohols to an aqueous-salt solvent exert a stabilising influence on the native structure of many other proteins, too [31–38]. The experimental data are evidence of a non-specific effect of alcohols. It is noted in these works that the stabilising action of alcohols has an indirect character due to stabilisation of the water structure. These effects can be explained by the preferential interactions of the cosolvents with the proteins; i.e., the protein stabilizers are preferentially excluded from the proteins, while the destabilizers (for example, urea) bind to them [31, 34–38].

It is of interest that Frisman *et al.* [39], on studying the role of water structure in the process of the radiation damage to DNA, have shown that, as the water structure becomes more stable due to ethanol addition (until the critical concentration), the conformational damages in the DNA are decreasing and, finally, at some concentration of the alcohol in the irradiated solution the damages disappear. Thus, in the system studied, the water structure stabilization in the presence of low ethanol concentrations also leads to the increase of stability of the DNA structure [39].

At ethanol concentrations of 15–25 vol.% in mixed solution the Ca^{2+} ions concentration required for DNA compactisation decreases (Fig. 3). This may be explained by the increase of the binding constants of counterions (Na^{+} and Ca^{2+}) and of the fraction of the DNA charge neutralized due to additional counterion condensation on lowering of solution ε value. Besides, the shifts of absorption bands 1053→1056 and 1223→1225 cm^{-1} in the IR spectra of DNA without Ca^{2+} ions in mixed solution containing 25 vol.% of ethanol may be evidence of insignificant destabilisation of the DNA structure in solution. As a result, the DNA structural transitions under action of Ca^{2+} ions could become easier.

For comparison, in the present work a study of the ethanol effect on Ca^{2+} interaction with polyphosphates in mixed solutions was carried out. Under the action of Ca^{2+} ions polyphosphates (pP) undergo structural transitions similar to the processes of DNA compactisation and aggregation (depending on the ratio of $[Ca^{2+}]/[P]$). The pP interaction with Ca^{2+} ions in aqueous and mixed solutions will be discussed in detail in another paper

[1]In our experiment the Ca^{2+} ion concentration necessary for DNA compactisation increased at lower ethanol concentration in solution than did the drop of the DNA [η] as the work [28] shows. But it should be taken into account that, unlike in the publications [28, 29], in the present work a solution with high DNA concentration was used. So, the effective ethanol concentration in solution should be considered, taking into account water bound to DNA.

(Hackl *et al.,* in preparation). In the present work we consider only briefly the effect of ethanol on the Ca^{2+} ions concentrations which induce the pP structural transitions. Ca^{2+} ions concentration required to induce the structural transitions in pP decreases practically linearly with the rise of the ethanol content in mixed solution (Fig. 3, curve 3). This may be explained by amplification of the counterion screening action on the charges of the pP phosphate groups due to the increase of the binding constants, and additional counterion condensation on polyelectrolyte on lowering of the solution dielectric permeability. Dependence of C_{init} on ethanol concentration in solution for pP-Ca^{2+} ions complexes, in contrast to the analogous dependence for DNA–Ca^{2+} ions complexes, is of monotonous character. Their results obtained permit to conclude that the solution structure has a stronger influence on the structural transitions of high-molecular DNA. Perhaps, this is due to stronger hydrophobic interactions present in DNA molecules, as compared with polyphosphates. It is of interest that Veselkov & Frisman [29], on the basis of results of investigations of the polymethacryl and polyacryl acids in solutions containing different alcohols, draw the conclusion that, at ethanol concentrations in solution corresponding to the disruption of the spatial structure of water, some peculiarities are observed in the behaviour of those molecules whose hydrophobic interactions are essential.

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